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Serum Prolactin and TSH in an In Vitro Fertilization Population: Is There a Link Between Fertilization and Thyroid Function?

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***Purpose*:** Measurements of TSH and prolactin are generally included in the evaluation of female infertility, but their value in women coming to in vitro fertilization (IVF) has been questioned.

***Methods*:** In this study, we sought to investigate whether prolactin or TSH, measured in 509 specimens collected prior to therapy, predicted outcome in a prospective study of couples undergoing IVF between 1994 and 2001.

***Results*:** TSH was higher in women whose fertility problem was attributed to a male factor, and prolactin was lower if the measurement was taken during menses. TSH and prolactin were positively correlated ($p < 0.0001$). Neither TSH nor prolactin levels correlated with overall IVF outcome; however, TSH levels were significantly higher among women who produced oocytes that failed to be fertilized and this finding persisted after adjustment for several covariates, including sperm motility. Among women who had a least one oocyte inseminated, the likelihood that they would have fewer than 50% of their eggs fertilized was significantly related to higher TSH levels in a multivariate model.

***Conclusion*:** We conclude that TSH may predict poor fertilization in IVF and reflect the importance of thyroid hormones in oocyte physiology.

KEY WORDS: Fertilization; IVF; oocytes; prolactin; TSH.

INTRODUCTION

Traditionally, measurements of prolactin and TSH have been considered important components of the evaluation of women presenting with infertility. In a survey of reproductive endocrinologists in the United States, more than half indicated that they "always" or "almost always" ordered TSH and prolactin to evaluate the endocrine status of their patients (1). However, the value of routine endocrine testing in an infertility population (2) and especially an in vitro fertilization (IVF) population has been questioned (3,4). In this study we sought to identify whether IVF outcomes were associated with pretreatment TSH and prolactin measured in archived blood specimens from women seeking IVF.

Patients and Methods

Since 1994, we have been studying couples undergoing assisted reproductive technology (ART) at

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three IVF clinics in greater Boston (Boston IVF, the Brigham and Women's IVF Program, and the Reproductive Science Center of Boston) under protocols approved by the Brigham and Women's Hospital Human Research Committee. The study has had two funding phases—the first operating between August 1994 and March 1998 and the second beginning January, 1999 and still ongoing. During both phases, we used self-administered questionnaries to obtain information on epidemiologic variables and abstracted details on treatment and outcome from clinic records. Treatment details included description of the controlled ovarian stimulation regimen that involved gonadotropin releasing hormone agonists used in short or long regimens and gonadotropins, either human menopausal or recombinant, as well as details related to retrieval, fertilization, and embryo transfer. Because we sought to identify epidemiologic characteristics of the couple that might predict IVF success, we excluded couples who required either donor eggs or semen and those serving as gestational carriers.

We also sought to obtain a variety of specimens in the women agreeing to be studied. This included, when possible, a basal blood specimen measured sometime during days 1 through 5 of a menstrual cycle prior to any use of hormones. For women in whom it was not possible to obtain a blood specimen timed to the menses, we sought to obtain a specimen before treatment was begun. The former specimens were called "true baseline" and the latter "initial" specimens. Additionally, we retrieved sera from bloods obtained during the course of ovarian stimulation, follicular fluid at the time of oocyte retrieval, and a luteal phase blood specimen. All specimens were processed, aliquoted, and stored at -80° C.

Approximately 65% of subjects approached in the first phase of the study agreed to participate and 1244 couples were enrolled. In the second phase of the study, 67% of couples approached agreed to participate and 974 had been enrolled as of July 2001. For the current investigation, we focused on women who had either a true baseline or an initial specimen obtained prior to any treatment and whose outcome for at least the first cycle of IVF was known. This yielded a total of 313 women from study 1 and 196 from study 2 with pretreatment specimens (true baseline or initial). In these 509 women, 44.8% were known to have had prolactin measured prior to enrollement and 38.5% were known to have had a prior TSH measurement.

Prolactin and TSH were measured in serum using the AxSYM Immunoassay system (Abbott Diagnostics, Chicago, IL). Both tests are solid-phase double antibody enzyme immunoassays employing microparticle enzyme immunoassay (MEIA) technology. Prolactin, captured by a monoclonal mouse antibody attached to microparticles, is measured by a rabbit antibody conjugated to alkaline phosphatase. This assay has no detectable cross-reactivity with FSH, LH, TSH, or hCG, $<0.01\%$ cross-reactivity with human Placental Lactogen and $<1.7\%$ cross-reactivity with hGH. Prolactin levels in serum were quantified (ng/mL) based on the basis of assay calibrators standardized using the World Health Organization 3rd Internationl Standard (WHO 3rd IS 84/500; 1 ng $= 24\ \mu$IU). The limit of detection (e.g., the lowest measurable concentration of prolactin in serum that can be distinguished from zero) was 0.6 ng/mL. The working range of the assay was from 0.6 to 200 ng/mL and performance of the assay was monitored using three quality control sera (Abbott Diagnostics). The mean concentrations of prolactin in control sera were 7.0, 17.6, and 34.8 ng/mL, and the CVs were 8.3, 6.8, and 4.8%, respectively.

Human TSH was also measured in serum by using the MEIA technology (Ultrasensitive hTSH II). TSH, captured by a monoclonal mouse antibody attached to microparticles, is measured by a goat antibody conjugated to alkaline phosphatase. This assay has <0.001 cross-reactivity with FSH and $<0.0001\%$ cross-reactivity with LH or hCG. TSH levels in serum were quantified (μIU/mL) on the basis of assay calibrators standardized using the World Health Organization TSH 80/558. The limit of detection (e.g., the lowest measurable concentration of TSH in serum that can be distinguished from zero) was 0.03 μIU/mL. The functional sensitivity (concentration of hTSH that can be measured with an interassay CV of 20%) was 0.06 μIU/mL. The working range of the assay was from 0.1 to 100 μIU/mL. Performance was monitored using three quality control sera (Abbott Diagnostics). The mean concentrations of hTSH in the controls were 0.26, 6.14, and 29.72 μIU/mL, and the CVs were 7.1, 6.2, and 7.4%, respectively.

Means and standard deviations for TSH and prolactin were examined in subjects categorized by various baseline characteristics as well as categorical outcomes including whether a clinical pregnancy was achieved, at what point failure during the IVF process may have occurred, or by average or below average oocyte fertilization. We chose to focus on first cycle results because this would yield the outcomes closest in time to when the pretreatment blood had been drawn. Differences among women by level of TSH or prolactin for particular characteristics were assessed by

analysis of variance followed by the Bonferroni test to assess pairwise differences. Pearson correlations were calculated when the hormone values were compared with continuous outcomes such as estradiol level during treatment, the number of oocytes retrieved, and the fertilization rate. The fertilization rate was defined as the number of oocytes with two pronuclei after insemination divided by the number of oocytes inseminated and was restricted to those who had at least one oocyte inseminated. To adjust for potential confounding factors, generalized linear or unconditional logistic regression modeling was used depending on whether the outcome variable was continuous or dichotomous. To account for skewness generally present in hormonal data, log transformation of the hormonal variables was used for significance testing, although arithmetic means and standard deviations are tabulated.

RESULTS

Table I shows means (and standard deviations) for prolactin and TSH by subject characteristics.

Table I. Mean Hormone Levels by Characteristics of Subjects or Specimens

		Prolactin (ng/mL)		TSH (μIU/mL)	
	Number	Mean	SD	Mean	SD
Age					
<30	45	15.6	10.5	1.9	1.3
30–34	175	16.0	8.6	2.3	4.4
35–39	200	15.9	9.2	2.0	1.2
>39	89	16.3	9.5	2.2	1.8
p value		0.82		0.68	
BMI					
<20.4	100	16.2	9.3	1.9	1.2
20.4–21.6	99	16.1	10.8	1.9	1.6
21.7–23.4	103	15.8	8.4	1.7	0.8
23.5–26.2	100	16.9	8.9	2.8	5.5
≥26.3	101	14.8	8.3	2.3	2.3
p value		0.37		0.17	
Primary diagnosis					
Male	92	16.1	8.8	3.0	5.8
Ovulatory	54	14.2	8.5	1.8	1.1
Tubal	118	16.1	9.1	2.0	2.1
Endometriosis	74	16.9	8.6	2.0	1.2
Unexplained/other	166	16.0	9.9	1.9	1.2
p value		0.39		0.003	
Study					
1	313	15.6	8.6	2.3	3.5
2	196	16.6	10.0	1.9	1.0
p value		0.24		0.60	
Specimen					
Menstrually timed	233	14.6	7.0	2.0	1.5
Other	286	17.1	10.4	2.3	3.5
p value		0.03		0.21	

No statistically significant differences in TSH or prolactin were observed for categories of age or BMI. Significant variation was observed for TSH and primary infertility diagnosis. Curiously, women whose infertility had been attributed to a male factor had significantly higher TSH levels than did any other category of infertility. Additional adjustment for age did not negate this association. No significant variation was noted between study 1 and study 2 by level of prolactin or TSH. However, women whose blood specimen had been drawn during the menstrual phase of their cycle had significantly lower prolactin levels than did women whose specimens had been drawn at other times during the cycle.

Table II shows Pearson correlation coefficients between prolactin, TSH, and some continuous outcome variables observed during the IVF cycle. A predictable correlation included the strong positive correlation ($r = 0.719$) between the number of oocytes retrieved and the estradiol level prior to hCG administration ($p < 0.0001$). TSH and prolactin were also positively correlated ($r = 0.185$) with a high degree of significance (<0.0001) but with a relatively weak correlation. TSH and prolactin were weakly correlated with the estradiol level (borderline significance) but not with the number of oocytes retrieved. No significant correlations were noted with the fertilization rate. Additional adjustment for female age in a general linear model did not negate the significant associations observed or improve the ones of borderline significance including the correlation

Table II. Correlation Coefficients Among Hormones Continuous IVF Outcomes in First Cycle[a]

	TSH	Pre-HCG estradiol	Oocytes retrieved	Fertilization rate[b]
Prolactin				
N	505	496	484	446
Correlation	0.185	0.074	0.023	−0.053
p value	<0.0001	0.101	0.611	0.266
TSH				
N		492	480	443
Correlation		0.080	0.043	−0.073
p value		0.075	0.348	0.123
Estradiol				
N			474	442
Correlation			0.634	−0.045
p value			<0.0001	0.347
Oocytes retrieved				
N				446
Correlation				−0.065
p value				0.170

[a] Based on log transformed hormone values.
[b] Defined as number of oocytes with two pronuclei after insemination divided by the number of oocytes inseminated.

Table III. Mean Hormone Levels by First Cycle Pregnancy Outcomes

		Prolactin (ng/mL)		TSH (μIU/mL)	
	Number	Mean	SD	Mean	SD
Clinical pregnancy					
Yes	151	15.9	8.6	2.2	2.2
No	358	16.0	9.4	2.1	3.1
p value		0.78		0.21	
Detailed outcome					
Failed retrieval	50	14.8	9.4	1.8	1.2
Failed fertilization	22	17.2	8.2	5.1	11.6
Failed implantation	285	16.1	9.5	1.9	1.2
SAB	22	16.3	10.7	2.7	3.0
Liveborn	126	15.7	8.2	2.1	2.1
p value		0.721		0.004	
Fertilization rate					
<50%	141	16.6	8.6	2.5	4.7
≥50%	305	15.7	9.1	2.0	1.7
p value		0.23		0.05	

between TSH and the fertilization rate (data not shown).

Table III shows prolactin and TSH levels by dichotomous outcomes for IVF. Mean levels of prolactin and TSH did not differ between women who did or did not achieve a clinical pregnancy during their first cycle of IVF. Even when all cycles were included, level of TSH or prolactin did not predict who ultimately achieved a clinical pregnancy (data not shown). However, when we examined the specific point of failure during the first IVF cycle, women who had oocytes retrieved but failed to have embryos developed for transfer had a significantly higher TSH level. Six subjects (25%) with failure of fertilization had a TSH level above 3.9 μIU/mL; two among these were on what was likely an inadequate dose of thyroid supplementation. The last entry in Table III examines hormone levels in women stratified by the percentage of their oocytes successfully fertilized. Women with "poor" fertilization, defined as having fewer than 50% of inseminated oocytes proceeding to the two pronuclei stage, had significantly higher TSH levels than did women with 50% or more of their oocytes successfully fertilized.

We next used unconditional logistic regression analysis to examine whether TSH persisted as a predictor of fertilization failure or poor fertilization after adjustment for a number of covariates. TSH continued to be a significant predictor of fertilization failure in a logistic model that adjusted for age of the woman, year of enrollment, BMI category, infertility diagnosis, type of specimen, number of oocytes retrieved, and sperm motility (which we identified as the strongest male determinant of fertilization failure). Only one subject in the fertilization failure group had had ISCI, yielding unstable estimates of its effect in the model. We used these same variables in a logistic model to predict poor fertilization and again found TSH remained a significant predictor of poor fertilization (Table IV). About 26% of subjects in both the poor or normal fertilization groups had intracytosplasmic sperm injection (ICSI) and this variable did not affect the estimate of TSH in the fertilization model (data not shown).

Table IV. Logistic Model Examining the Role of TSH for Insemination Failure or Poor Fertilization

	Fertilization failure ($N = 22$)		Poor fertilization ($N = 141$)	
Variable	Parameter	Probability	Parameter	Probability
TSH	1.016	0.002	0.358	0.05
Age	0.007	0.90	0.008	0.75
Year enrolled	−0.242	0.09	−0.111	0.05
BMI category	−0.041	0.81	0.056	0.46
Specimen type	−0.131	0.79	−0.354	0.10
Sperm motility	−0.401	0.43	−0.440	0.05
Oocytes retrieved	−0.052	0.17	0.011	0.44

DISCUSSION

The relationships between thyroid hormones, TSH, prolactin, and female infertility are multiple and complex. Thyrotropin-releasing hormone is a potent stimulus of prolactin and the association between hypothyrodism and hyperprolactinemia is well appreciated. Hyperprolactinemia, because of a variety of causes, can reduce pulsatile GnRH secretion and interfere with ovulation (5). Even in the absence of hyperprolactinemia, hypothyroidism may contribute to infertility since thyroid hormone may be necessary for maximum production of both estradiol and progesterone (6). Conversely, most women with hyperthyroidism do not have fertility problems, although 25% may have irregular menses (7). For these reasons, TSH and prolactin are commonly ordered clinical tests in evaluating an infertile woman (1).

Because the deleterious effects of high prolactin or low thyroid seem likely to be mediated through effects on ovulation, it is less clear that correction of mild problems is necessary in an IVF population undergoing controlled ovarian stimulation. Thus, it has been argued that screening TSH and prolactin is unnecessary in an IVF population, especially in those with

fertility problems other than ovulatory (3,4). Among the women having prior TSH or prolactin measurements in our study, 26 subjects (5.1%) were on thyroid hormones and 1 was on a dopamine agoinst. While the primary purpose of our study was to look for subtle effects of prolactin or thyroid hormone (as inferred from the TSH level), it should be appreciated that some abnormal levels were encountered. Thus among the group who had no prior TSH performed, 10 (3.1%) subjects had a value over 5 μIU/mL including one with a value of 55 μIU/mL. Among the subjects who had not had prolactin measured, 20 (7%) had a value over 30 ng/mL. Related to routine testing, a finding of our study that has practical implication for testing was that prolactin measured during the menstrual phase of the cycle has a lower level than when it is measured at other phases of the cycle. It has been previously reported that prolactin levels are higher in the luteal phase (8).

Our study yielded other findings—both expected and unexpected. Perhaps not surprising, TSH is positively correlated with prolactin even over the largely normal range examined in this study. More surprising were our observations concerning the association between TSH and IVF outcome. Although neither TSH nor prolactin predicated whether a clinical pregancy resulted from IVF, TSH was correlated with the likelihood of fertilization. Women who had oocytes retrieved but failed to have any or enough successfully fertilized for transfer (i.e., recorded as a fertilization failure in Table III) had significantly higher TSH. This finding persisted after adjustment for a number of covariates, including age of the woman and sperm motility (which was the strongest of the sperm parameters influencing fertilization). As further evidence suggesting a link between TSH and fertilization, we found that TSH was a significant predictor of the likelihood that a woman would have fewer than 50% of inseminated oocytes successfully fertilized—a finding which also persisted after adjustment for covariates (Table IV).

In seeking a biologic explanation for this finding, we considered whether TSH itself might adversely affect fertilization. Studies have examined the effects of pituitary hormones on oocyte development in culture in a variety of species. Bovine oocyte maturation is enhanced by intermediate but not high doses of TSH, and LH was found to further enhance the maturation effect of TSH (9). In other species including monkeys, TSH does not enhance the effectiveness of gonadotropins on various measures of oocyte development, but neither does it appear to have a clear deleterious effect on its own (10–12). Thus it seems more likely that the association we observed between TSH and fertilization, if real, is an indirect one that reflects a relationship with thyroid hormone levels.

A number of thyroid receptor isoforms appear to be expressed in human oocytes, cumulus cells, and granulosa cells (13), and T4 induces estrogen and progesterone production from cultured granulosa cells (6). In *Xenopus*, T3 appears to be readily transported into oocytes (14), which also possess high sulfotransferase activity specific for the metabolism of T3 (15). In fish, T3 appears to be preferentially accumulated by oocytes and, together with gonadotropins, is able to trigger oocyte maturation in fish kept below spawning temperature (16). In hypothyroid rats, both gonadotropins and thyroxine appear to be necessary to achieve maximum fertilization rates and blastocyst development (17).

Because we did not measure thyroid hormones in this study addressing simple screening tests, our hypothesis that the association between TSH and fertilization reflects thyroid hormone levels remains a matter of speculation. Clearly, we must also consider the possibility that the association is a chance finding or reflects some other characteristic of women with mildly elevated TSH that is linked with fertilization problems. Regarding the latter issue, we note that we did adjust for a number of factors including female age, BMI, oocyte number, as well as sperm motility, yet the association persisted. To address the issue of chance, confirmation in another data set will be necessary, although we note that the associations between fertilization failure or poor fertilization went in the same direction in both the earlier and more recent phase of the study included here.

At first look, chance might seem a likely explanation for the surprising finding that women whose primary fertility problem had been attributed to male factor had higher TSH levels; however, there is an alternate explanation. Since, in our study, couples who required donor semen were excluded, the "male infertility" group consisted of men with oligospermia or athenospermia rather than azospermia. For some time, it has been appreciated that female factors also likely operate in couples with an oligospermic male as inferred from data on donor insemination. Women married to oligospermic men have poorer age-specific fertility rates after donor insemination than do women married to azospermic males (18,19). Thus, in searching for other data sets to address the association between fertilization and thyroid function, data from the insemination clinic may also be useful.

In summary, we have identified the novel association among women undergoing IVF that TSH levels are inversely related to fertilization. If real, the association likely reflects the importance of thyroid hormones for successful oocyte maturation and fertilization. The findings of this study, combined with evidence that high maternal TSH during pregnancy may correlate with poorer neurologic development of the infant (20), provide a strong rationale for TSH screening in an infertile population seeking therapy. At the very least, women with unexplained insemination failure or poor fertilization should have TSH testing performed. Precise clinical recommendations about the threshold level to begin thyroid supplementation best await further study and might be appropriately addressed by a clinical trial in an IVF population with marginally elevated TSH levels.

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